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Metabolism of ^{14}C -labelled Insecticides in Microorganisms, Insects and Mammals

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綜 説

Metabolism of ^{14}C -labelled Insecticides in Microorganisms, Insects and Mammals.

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Following survey is summarized experiments since 1958 on the conversion of some drin-insecticides in living organisms under comparable conditions. To this aim, we had first to synthesize these insecticides with high specific activity, starting from barium-carbonate- ^{14}C . For our metabolism studies we have now synthesized ^{14}C -labelled aldrin¹⁾, dieldrin¹⁾, endrin⁵⁾, telodrin³⁾, heptachlor⁴⁾, dihydroheptachlor²⁾ and chlordane⁴⁾. A survey of these compounds is given in Fig. 1.

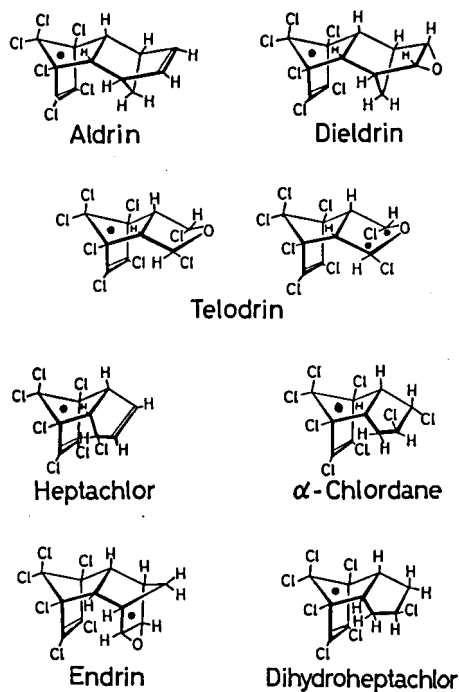


Fig. 1.

The circles in the structural formulae indicate the position of the ^{14}C -labelled atoms. Telodrin was synthesized with the label in 1,3-position, all others are labelled statistically in the hexachlorocyclopentene ring-system with the exception of endrin, where the ^{14}C -atom is placed in the cyclopentene-ring. Besides well-known insecticides

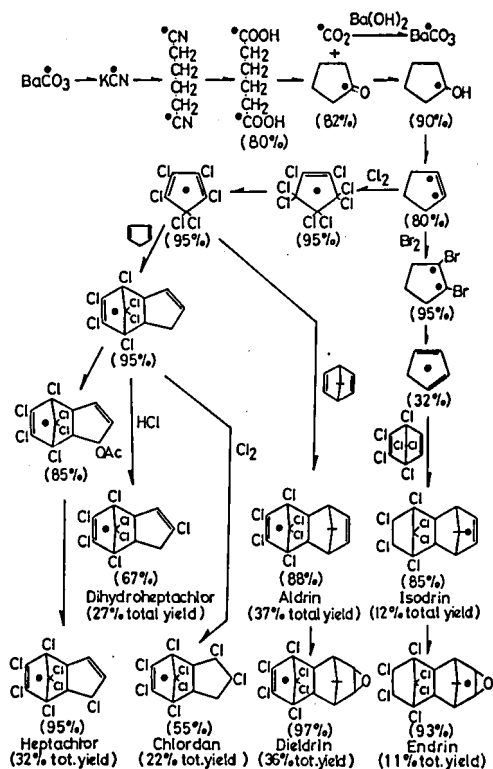
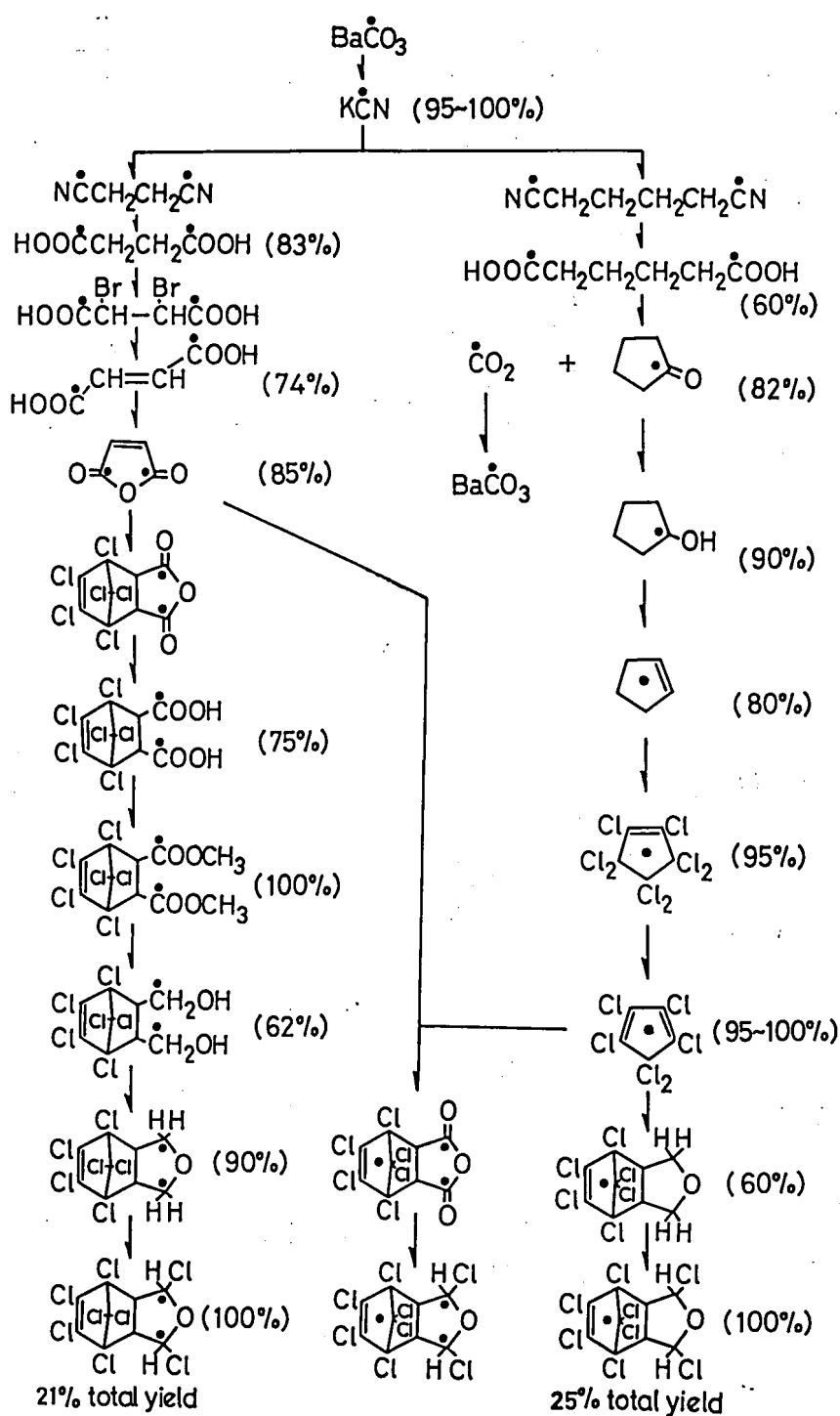


Fig. 2. Synthesis of aldrin- ^{14}C , dieldrin- ^{14}C , chlordane- ^{14}C , heptachlor- ^{14}C , dihydroheptachlor- ^{14}C and endrin- ^{14}C .



like aldrin, dieldrin, endrin etc. we also included one compound of this group which has a surprisingly low mammalian toxicity of more than 5,000 mg/kg mice orally, namely the dihydroheptachlor. In Fig. 2. the routes followed in some of our microsyntheses are demonstrated.

Aldrin, dieldrin, heptachlor, dihydroheptachlor and chlordane were obtained by condensation with ^{14}C -labelled hexachlorocyclopentadiene which was synthesized in the following manner: Starting from barium carbonate- ^{14}C and *via* K^{14}CN we obtained adipic acid-1,6- ^{14}C which after decarboxylation gave cyclopentanone- ^{14}C . From this we obtained the hexachlorocyclopentadiene- ^{14}C *via* cyclopentanol- ^{14}C , cyclopentene- ^{14}C and octachloropentene. Isodrin and endrin were obtained by condensation of hexachlorobicycloheptadiene with cyclopentadiene- ^{14}C , which was synthesized from cyclopentene- ^{14}C *via* dibromocyclopentane- ^{14}C .

In the syntheses of telodrin- ^{14}C we used the route *via* hexachlorocyclopentadiene- ^{14}C (shown on the right hand side of Fig. 3.) and also developed a synthesis for labelling in 1, 3-position: Again starting with barium carbonate- ^{14}C , we obtained *via* K^{14}CN succinic acid-1, 4- ^{14}C and from this, *via* fumaric acid, the maleic acid anhydride-1, 4- ^{14}C . Diels-Alder-condensation with hexachlorocyclopentadiene and subsequent hydrolysis gave the dicarboxylic acid from which, *via* intermediate steps shown in Fig. 3, telodrin-1, 3- ^{14}C was obtained in a total yield of 21%. The centre of this figure shows a possible synthesis route for a product with extremely high specific activity.

Once we disposed of the ^{14}C -labelled compounds, we first examined whether these chemicals would be degraded by living organisms, and if so to which extent, and secondly what kind of metabolism products would result from such a conversion.

For a start, we used for these studies fungi, *Aspergillus niger* and *flavus* as well as *Penicillium notatum* and *chrysogenum*.

In each case the ^{14}C -labelled drin-insecticide was applied to the growing fungus culture. After the cultures had reached full growth, the mycelium and the culture medium were worked up separately. Except for dieldrin where no conversion could be detected, we found in each case con-

siderable amounts of metabolism products in both the culture medium and the mycelium. Their behaviour in chromatography indicated that these metabolism products are more hydrophilic than the toxicant applied. From ordinary soil we isolated a fungus which grows on a medium containing large amounts of aldrin. The Centraalbureau for Schimmelcultures at Baarn identified this specimen as *Penicillium vermiculatum* (Dangeard). Fig. 4. illustrates the results of these experiments on the example of *Aspergillus flavus* after a certain period of incubation.

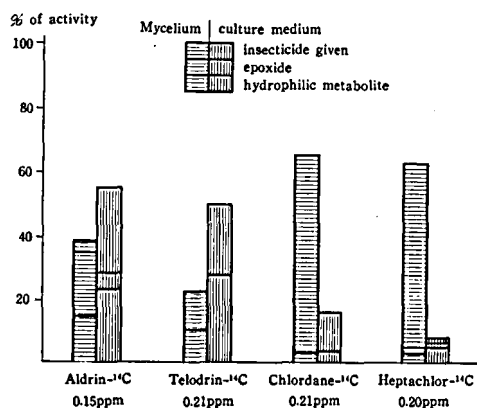


Fig. 4. Metabolism of ^{14}C -labelled insecticides (*Aspergillus flavus*).

The cross-striped columns show the radioactivity found in the mycelium, while the vertical lines indicate the radioactivity in the culture medium.

Generally speaking, it can be said that the percentage of metabolism products increases in inverse proportion to the concentration of the insecticide added. Furthermore, as we can see from Fig. 4, there is a higher percentage of metabolism products in the aqueous phase than in the mycelium.

Our next experiments were carried out with mosquito larvae (*Aedes aegypti*)^{6,7}. We let the larvae develop in an aqueous culture medium to which the drin-insecticide was added. Generally speaking, we can say that under the conditions employed all these insecticides—including dieldrin!—are converted to metabolism products at a rate of 10 to 25 per cent. The percentage of metabolism products increases with lower concentration of insecticide and larger amounts of larvae used.

Since it is known that drin-insecticides are unstable under ultraviolet light and rearrange to bird-cage and half-bird-cage derivatives⁹⁾, when heated over 150°C, and that they also can be transformed by air while being absorbed on active surfaces, it was, of course, necessary to show that our products were not artificial ones, but that conversion was indeed effected by the living cells. The following two results clearly point to natural metabolism products: **Firstly**, when working under comparable conditions with living larvae and with their homogenates, the latter did not lead to any conversion, while metabolism took place in the experiment with living cells, **Secondly**, when the larvae were kept in contact with dieldrin for 24 hours and were subsequently separated from the aqueous phase, qualitative and quantitative determination of the radioactivity contained in the larvae showed that it consisted of unchanged dieldrin. Now these larvae containing only the dieldrin itself were put into fresh water for further 24 hours. We could then prove that they had excreted hydrophilic metabolites into the aqueous phase and themselves contained up to 40 per cent of these metabolites. This confirms the observations already made in the homogenate test and shows that the dieldrin in the living larvae was converted to metabolism products and were excreted as such. We have, of course, made control runs for all tests in order to exclude the possibility of exterior physical influences. In none of these control experiments could any conversion be observed.

Our attempts to isolate and identify a metabolite from mosquito larvae were successful in the case of telodrin⁷⁾. We found a compound which after hydrolysis gave a lactone that had already been synthesized and described by Riemschneider⁹⁾ some time ago. This simply means that the chlorine atoms in 1,3-position of the telodrin are split off by hydrolysis, leading to the hydroxy acid which closes to the stable lactone with the same configuration as telodrin, which is shown in Fig. 5.

As shown in Table 1, mammalian toxicity of the lactone is 30 times, insect toxicity (tested on *Aedes aegypti* larvae) 500 times lower than that of telodrin. Also in the case of mosquito larvae

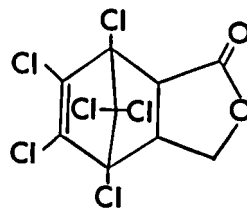


Fig. 5.

Table 1. Mammalian toxicity (mice)
LD₅₀ (mg/kg)

	Oral	Intravenous
telodrin	9.5	2.9
lactone I	306	100
aldrin	95	21.5

all metabolites, or at least the main conversion products, were clearly characterized by chromatography. After the detection of hydrophilic metabolites in fungi and mosquito larvae, it seemed certainly of great interest to study whether mammals are also able to metabolize and detoxify these insecticides. Therefore we undertook experiments with rats and rabbits¹⁰⁾.

When we started these investigations no published results on studies of metabolism of drin-insecticides by animals were available, and we believed that these insecticides were relatively stable and not easily metabolized. We were, therefore, much surprised to find that the radioactive compounds isolated from the excreta already one day after intravenous application consisted mainly of metabolites. Dieldrin was converted by rats and rabbits also to products which, in view of their behaviour in chromatography, must be more hydrophilic than the insecticides applied, and which are probably identical with those obtained from aldrin. After 48 hours we sacrificed the animals of each experiment in order to check the distribution of radioactivity in the entire body and found in the greater part of the organism unchanged insecticide, while the excretion organs like kidney and liver as well as the gut contained a very high percentage of hydrophilic product, as can be seen in Fig. 6 on the example of dieldrin.

Similar results were obtained by the experiments

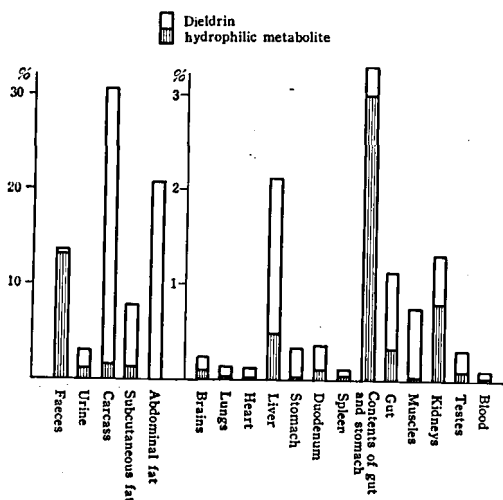


Fig. 6. Distribution and excretion of radioactivity in rats 48 hours after intravenous injection of $19\mu\text{g}$ dieldrin- ^{14}C .

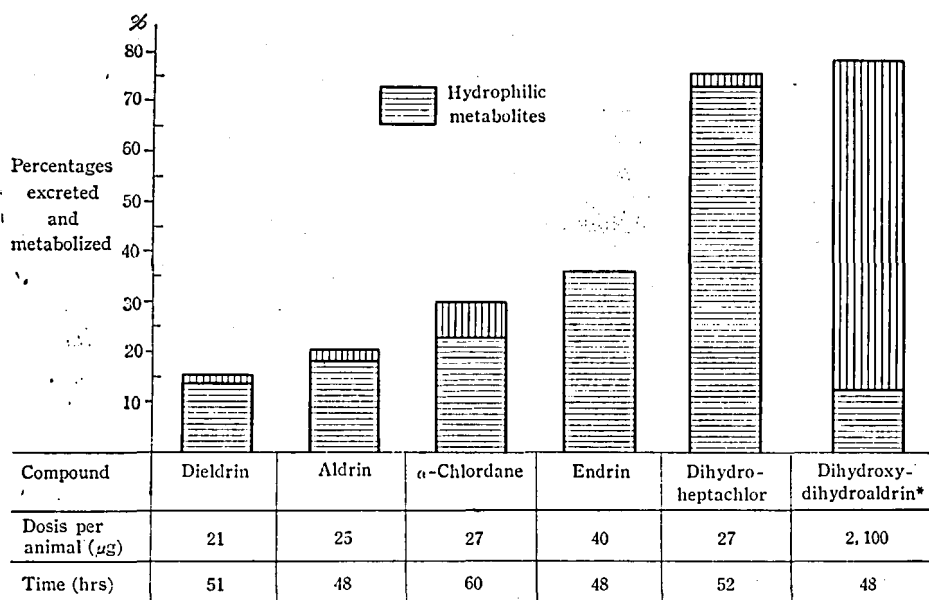
with the other drin-insecticides investigated. Fig. 7 shows a survey of the excreted and metabolized amounts of the different insecticides, as found during these investigations.

The columns represent the percentage of total excreted radioactivity, the cross striped part of the column indicates the percentages of hydrophilic

metabolites excreted. The relatively high excretion and metabolism rate of endrin seems remarkable. The low metabolism rate of the dihydroxy-dihydroaldrin is easily understood, as this compound is the main metabolite of dieldrin which was found to be chiefly excreted in unchanged state.

Knowing that microorganisms are able to convert insecticides to metabolites, the next step then was to find out whether conversion in the mammal is effected by microorganisms in the intestinal tract, or whether the conversion actually occurs in the animal organism itself. In bile fistula experiments the insecticide was applied intravenously and the radioactivity was measured and analysed directly after leaving the bile duct¹⁰. The results are graphically shown in Fig. 8. It was proved that a maximum of secretion occurred within one hour after the injection, in the case of both aldrin and dieldrin. Four hours after the injection of aldrin- ^{14}C , 16 per cent of total injected activity had been excreted *via* the bile, mainly as hydrophilic metabolism product.

The results of analyses of the bile secretion and of the different tissues at the end of the experiment (after 4.5 hours) are graphically



Compound	Dieldrin	Aldrin	α-Chlordane	Endrin	Dihydro-heptachlor	Dihydroxy-dihydroaldrin*
Dosis per animal (μg)	21	25	27	40	27	2, 100
Time (hrs)	51	48	60	48	52	48

*Dieldrin metabolite

Fig. 7. Excretion and metabolism of drin-insecticides by male rats (Wistar) after intravenous administration.

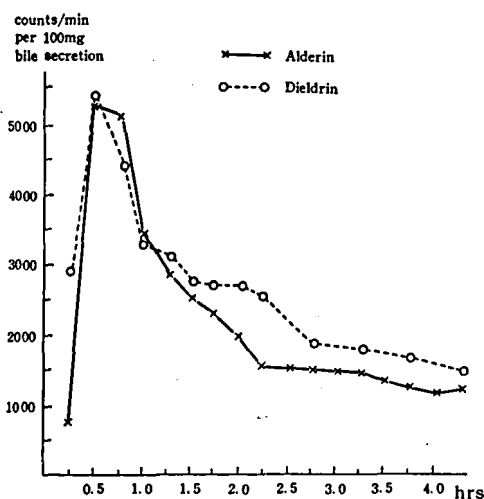


Fig. 8.

shown in Fig. 9. As can be seen, the bile secretion contained up to 62% of hydrophilic products (the part of the vertically striped columns). In the case of dieldrin, the fractions of the bile secretion contained even up to 90~95% of hydrophilic

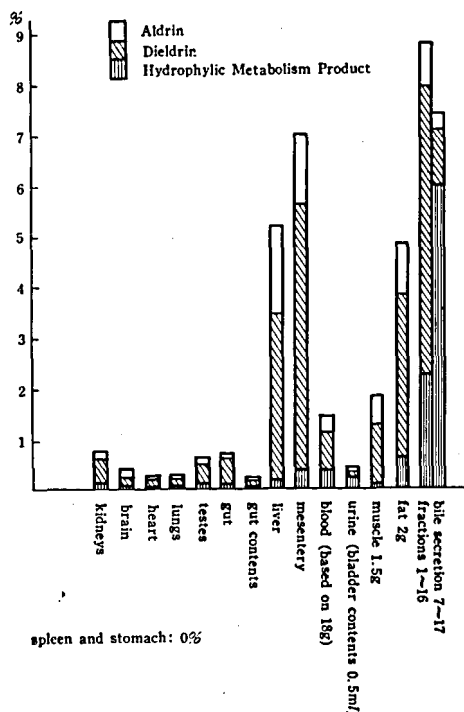


Fig. 9. Analysis of tissues and bile secretion of a rat after injection of 46 μg aldrin- C^{14} (Weight of rat:230g.)

products.

Analogous experiments with telodrin, chlordane and heptachlor- C^{14} showed that also in these cases the bile secretion contained predominantly hydrophilic products.

Furthermore we carried out perfusion experiments of the liver of rats with blood containing aldrin- C^{14} . After the blood had twice passed the liver, only about 15% of the total radioactivity were found remaining in the blood, consisting of about 65% of dieldrin and 35% of unchanged aldrin. About 85% of total radioactivity were found in the liver which contained only about 25% of the radioactivity as unchanged aldrin, while 75% were found to be epoxydized to dieldrin. 1% of the total activity applied could be obtained from the ductus hepaticus, again as hydrophilic metabolism products.

These two experiments clearly demonstrate that conversion takes place in the animal organism and is not caused by microorganisms in the intestinal tract.

While in our first experiments with mammals the insecticide was applied intravenously, we now wanted to study whether and in which quantities metabolism products would be found, if the insecticide were given orally¹¹. Therefore, a constant daily amount of insecticide—corresponding to twice the U.S. tolerance dose for residues on potatoes—was given to male and female rats in the diet (0,2ppm). In the excreta of these rats we found the same metabolites as in the case of intravenous application. The main metabolite found in the urin obviously differs from the one found in the faeces. Each day we collected urine and faeces and quantitatively and qualitatively analyzed the radioactive compounds isolated. The result of this work for the male animals is graphically shown in Fig. 10. The uninterrupted lines represent mean values of 4 animals, while each of the other lines represents mean values of 2 animals.

After 50 days, approximately the entire activity applied daily was also daily excreted, which means that a saturation level was reached at that stage in the male rats under investigation. Subsequent oral doses did not lead to a higher concentration of the total insecticide in the organism. Up to

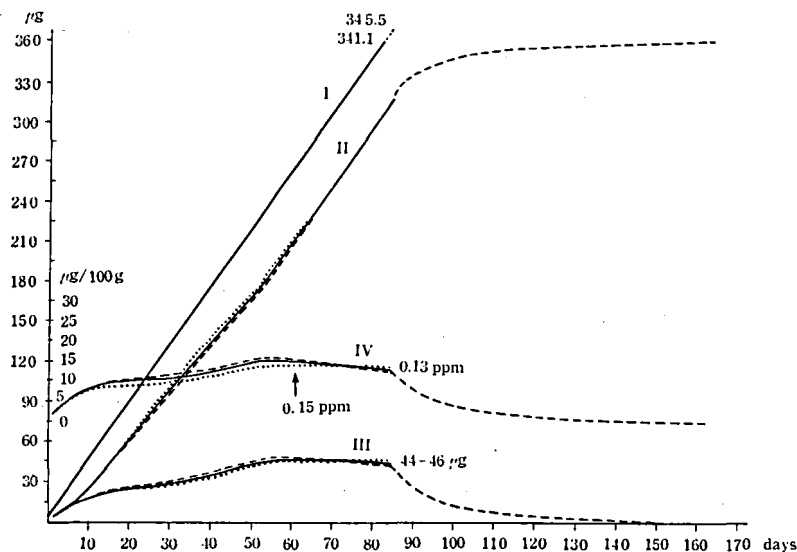


Fig. 10.

- I total aldrin- ^{14}C applied ($4.3 \mu\text{g}$ daily, 0.2 ppm in the diet)
 II total excreted amount
 III total stored amount
 IV concentration ($\mu\text{g}/100\text{g}$ weight) ($1 \mu\text{g}=0.01 \text{ ppm}$)
 mean values of 2 rats killed 24 hours after last dosing
 ---- mean values of 2 rats killed after 162 days
 — mean values of all 4 rats

70 per cent of the activity found in the faeces and up to 95 per cent of that found in the urine consisted of metabolites. After daily oral application had been stopped, we continued to control the excretion of radioactivity. We thus obtained a declining curve which showed that half of the activity present in the organism had been excreted—mainly as metabolites—after 10 days, and three quarters after 21 days.

At the same feeding level the rate of excretion of female rats proved to be lower than that of the male rats studied. Therefore the level of saturation was reached only after about 200 days of daily feeding and—within the range of biological variation—remained constant during the rest of the feeding period (55 days). After termination of daily administration, the declining curve was much flatter than that obtained for the male rats, and thus the biological half-life-period was found to amount to about 100 days for female animals.

Analyses of the excreted activity gave about the same percentage of hydrophilic metabolites as found in the excreta of male rats: Up to 70

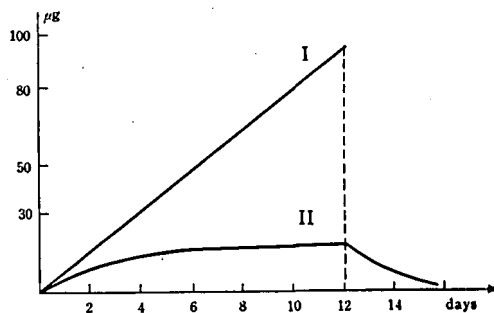


Fig. 11.

- I = total endrin- ^{14}C applied
 II = total stored amount

percent in the faeces and up to 95 per cent in the urine. This means that the female rats also do metabolize the insecticide, though metabolism and excretion rate are lower than in the case of the male animals.

The results of appropriate experiments with endrin- ^{14}C ¹² is shown in Fig. 11. When endrin- ^{14}C is applied orally ($8 \mu\text{g}/\text{animal}$ and day) a saturation level is already reached after 9 to 10 days for male as well as for female rats. The main amount of activity is excreted in the faeces consisting of up to 80% of more hydrophylic metabolites and 20%

of endrin. After daily application had been stopped, control of the excretion of radioactivity gave a declining curve, which showed that half of the activity present in the organism had been excreted very quickly—after 1 to 2 days.

The results of these tests show the following:

- (a) When applying constant daily doses of the drin-insecticide, a saturation level is reached after a certain time (which has already proved in the case of DDT);
- (b) if daily oral application of aldrin- ^{14}C is stopped, a declining curve is obtained, which is characterized by a biological half-life period of 10 to 11 days for male animals, and 200 days for females, the biological half-life period for endrin is 1 to 2 days, and
- (c) the greater part of the excreted radioactivity consists of metabolism products and not of the insecticide applied.

The different behaviour of the two sexes was also observed during the following studies. Having fed male and female rats with a certain constant daily amount of aldrin- ^{14}C and having then sacrificed them after about 3 months, we analysed the different tissues of the animals separately. The results of analyses showed remarkable differences for lungs, liver, spleen and kidneys, which are demonstrated in Fig. 12. Qualitatively the amount of radioactivity found in the tissues of the female animals was about twice as high as that found in the tissues of the males. More impressive still are the quantitative results.

In this connection it might be of interest that the epoxidation of aldrin, isodrin and heptachlor

by rat liver microsomes has recently been studied *in vitro* by D. T. Wong and L. C. Terriere¹³⁾, who found considerable sex differences in the epoxidation of these insecticides. Conversion to epoxides by microsomes from male rats was found to be up to 80~85%, from female rats only 3~6%. These *in vitro* results also indicate that female animals metabolize aldrin, isodrin and heptachlor less rapidly than male animals.

The detection of hydrophilic metabolites in the excreta of rats after intravenous and oral administration as well as in other biological organisms is certainly of some consequence for residue analyses of these insecticides. Our results were confirmed by other workers which also found hydrophilic metabolites in different organisms after administration of dieldrin: for instance by Cohen in locusts¹⁴⁾, by Gerolt in houseflies¹⁵⁾, and by Dahm and Hamilton in American cockroaches¹⁶⁾. Cueto¹⁷⁾ had reported on dieldrin metabolites in the human urine, and Heath¹⁸⁾ found after administration of dieldrin- ^{36}Cl water-soluble metabolites in the excreta of mice. But nothing was known on the chemical structure and the toxicity of such hydrophilic metabolites. Therefore we aimed at isolation and identification of these hydrophilic metabolites from the excreta of rabbits. So far, we have succeeded in isolating in crystalline form metabolites of α -chlordane- ^{14}C and dieldrin- ^{14}C .

Isolation and Identification of Metabolites of α -chlordane- ^{14}C

For the isolation of metabolites of α -chlordane¹⁹⁾, two healthy male rabbits—kept in individual metabolism cages—received weekly doses of 100mg of α -chlordane- ^{14}C for a period of 10 weeks by means of a stomach tube. Faeces and urine of each rabbit were collected at regular intervals for twelve weeks. At the end of the experiment—two weeks after the last dose of α -chlordane- ^{14}C —the animals were sacrificed, their carcass, viscera and other tissues were separated, homogenized individually and extracted. The extracts were submitted to qualitative and quantitative analyses. The results of the analyses of the radioactivity found in different tissues of the body and in the excreta are graphically shown in Fig. 13.

The columns represent the percentages of the total administered radioactivity. The striped part

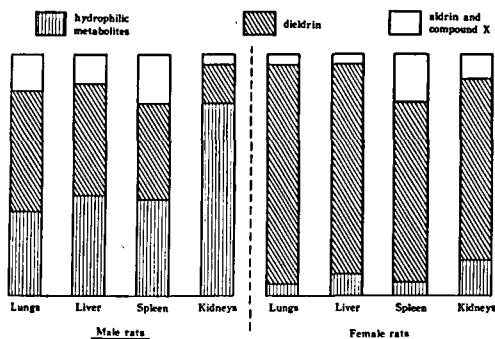


Fig. 12. Analyses of tissues of male and female rats after oral administration of aldrin- ^{14}C .

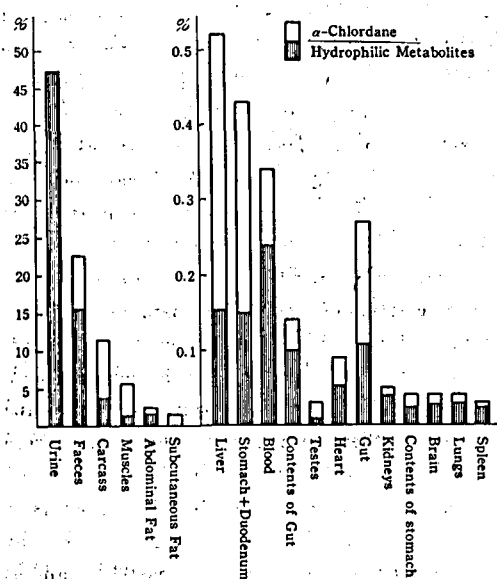


Fig. 13. Distribution and excretion of α -chlordane- ^{14}C and its metabolites by rabbits after oral administration (1,000 mg/per animal over a period of 10 weeks).

of the column indicates the amount of hydrophilic metabolites found in the extract, the white part the amount of unchanged α -chlordane- ^{14}C . The total recovery of radioactivity in this experiment amounted to 93 per cent.

The results clearly show that most of the radioactivity is excreted in urine and faeces, and that little is stored in fatty tissues. 47.2 per cent of the total administered activity were found to be excreted in the urine, 22.7 per cent in the faeces, while in the fatty tissues only about 4 per cent could be detected. As can be seen in Figure 13, unchanged α -chlordane was found only in the subcutaneous fat, while all other tissues investigated contained to a greater extent hydrophilic metabolites. In the urine only hydrophilic metabolites could be detected.

Thin layer chromatography of the extract of urine showed the presence of two radioactive substances, both of which were more hydrophilic than α -chlordane, which is demonstrated in Table 2.

The metabolites were purified and isolated by repeated thin-layer chromatography in different solvent systems. Metabolite A was obtained in crystalline form, and recrystallized from freshly

Table 2. R_f -values of metabolites of α -chlordane

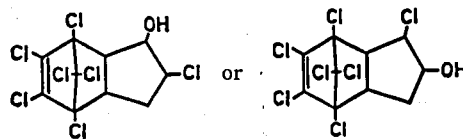
Mobil phase	Benzene	Benzene/ethyl-acetate (8:2)
α -chlordane	>0.95	>0.95
metabolite A	0.30	0.70
metabolite B	0.00	0.00

Thin-layer chromatography on silicagel G according to Stahl (E. Merck)

distilled cyclohexane.

The infrared spectrum of the metabolite A indicates the presence of at least one hydroxylic group, and the mass spectrum shows the presence of seven chlorine atoms and a molecular weight of 391. Elementary analysis of the metabolite A confirmed the molecular formula $\text{C}_{10}\text{H}_7\text{OCl}_7$ with a molecular weight of 391. This leads to the conclusion that in the metabolite one of the chlorine atoms of α -chlordane must be replaced in vivo by a hydroxylic group.

Therefore the formula for metabolite A could either be



which correspond to the formulae of the two isomers of chlorohydrine from chlordene. It was found that synthetical chlorohydrine of chlordene consisted of two compounds which were separated and purified chromatographically and recrystallized from cyclohexane. One of the purified chlorohydrines shows R_f -values identical to those of the metabolite A. The infrared and the mass spectra of this compound were also identical to those of metabolite A. This furnishes conclusive evidence that the metabolite A is identical with this compound of chlorohydrine.

The chemical structures of the two components of the synthetical chlorohydrine are shown in Fig. 14. The stereo chemical structures of chlorohydrines A and B were not taken into consideration.

In order to find out whether the hydroxylic group in metabolite A is in position 1 or 2, the four isomers Ia, Ib and IIa and IIb were compared

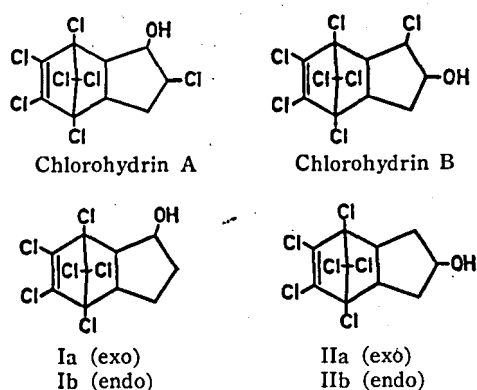


Fig. 14.

chromatographically.

Isomers IIa and IIb were found to be more hydrophilic than Ia and Ib. As chlorohydrine B was also more hydrophilic than chlorohydrine A, it can be assumed that in the chlorohydrine A the hydroxylic group must be in position 1 and chlorine in position 2. This establishes the structural formula of the metabolite A to be 1-hydroxy-2-chloro-dihydrochlordene.

A comparison of the analytical data of the metabolite A and the two components of chlorohydrine are summarized in Table 3. In metabolite B which was found to be more hydrophilic than metabolite A, both chlorine atoms in the position 1 and 2 of α -chlordane may be replaced by hydroxylic groups.

It was found that the acute oral toxicity of the metabolite A for mammals is lower than that of chlordane. The LD_{50} -value for mice is higher than 1,800mg/kg. Unfortunately a complete test was not possible due to insufficient amounts of substance. However, this value at least indicates that the toxicity of the metabolite must be less than about 1/3 of that of chlordane.

Isolation and Identification of Metabolites of Dieldrin- ^{14}C

For the isolation of metabolites of dieldrin²⁰, each of eight rabbits received 206mg of dieldrin- ^{14}C over a period of 21 weeks by means of a stomach tube, corresponding to a total of about 56~58mg/kg. The doses were administered twice per week. Single doses were increased from 2.5mg to 7.5mg during the experiment. The urine and faeces collected were extracted with diethyl ether.

At the end of the feeding period (22nd week) the animals had excreted in total 42.2% of the radioactivity administered (in urine 29.7%, in faeces 12.5%). After 52 weeks the excretion in the urine had increased to 43.1%, while the content of radioactivity in the faeces had decreased rapidly and was negligibly small from the end of the 24th week onwards.

The extract of the urine was prepurified by column chromatography on silicagel, which allowed separation of 6 different metabolites. Thin layer chromatography was employed for further puri-

Table 3. Comparison of analytical data of metabolite A and synthetical compounds.

	Metabolite A	Chlorohydrin A	Chlorohydrin B
Thin-layer chromatography			
R _F -Value a) I	0.30	0.30	0.20
II	0.70	0.70	0.50
Mass spectrum			
Molecular weight	391	391	
Number of Cl-atoms	7	7	
Infrared spectrum			
OH-Vibrations	3,380K	3,385K	3,270K
CH-Vibrations	2,915+2,940K	2,910+2,945K	2,940+2,950K
Elementary analyses b)			
% C	30.7	31.0	30.5
% H	1.7	1.8	1.6
% Cl	63.6	64.0	63.6

a) Silicagel G according to Stahl (E. Merck)

mobile phase: I. benzene- II. benzene/ethylacetate (8:2)

b) Calculated for $C_{10}H_7OCl_7$: molecular weight 391.4 30.7% C, 1.8% H, 63.4% Cl

fication of the compounds. The percentage distribution and the R_f -values of the six metabolites isolated are shown in Table 4.

Table 4. Percentage distribution and R_f -values of the dieldrin metabolites isolated from urine of rabbits.

Metabolite	I	II	III	IV	V	VI
Percentage	0.8	4.0	2.0	2.7	86.1	4.4
R_f -Value*	0.82	0.66	0.52	0.40	0.16	0.00

* Thin-layer chromatography on silicagel G according to Stahl from E. Merck—mobile phase: benzene/ethylacetate (3:1).

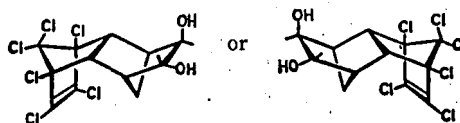
As can be seen from this Table, the main metabolite is present in amounts of about 86% of the total activity found in the urine. After purification in 9 different solvent systems, the metabolite V was obtained in needle-shaped crystals which were recrystallized from *n*-hexane and showed a melting point of 130~131°C. The mass spectrum showed the presence of 6 chlorine atoms and a molecular weight of 399. The infrared spectrum indicated the presence of hydroxylic groups. The results of the elementary analysis corresponded to the molecular formula $C_{12}H_{10}O_2Cl_6$ with a molecular weight of 399. As this formula contains one H_2O more than dieldrin, it was concluded that the epoxy ring system of dieldrin was hydrolyzed *in vivo* to the metabolite, leading to a 6,7-dihydroxydihydro-aldrin.

Comparison with 6,7-dihydroxydihydro-aldrin—which was obtained by oxidation of aldrin with $KMnO_4$ and which should have the two hydroxylic groups in *cis*-configuration—showed differences in melting point, infrared spectrum and R_f -values.

We synthesized the *trans*-6,7-dihydroxydihydro-aldrin by hydrolysis of dieldrin with H_2SO_4 . All the analytical data of the synthetical racemic *trans*-6,7-dihydroxydihydro-aldrin were in good agreement with those of the metabolite V, as can be seen from Table 5.

Later on it was found that the metabolite V is optically active with a specific rotation of $(\alpha)_D^{20} = -13.7$.

The structural formula of the metabolite V, therefore, must correspond to one of the two enantiomorphous isomers of synthetical racemic *trans*-6,7-dihydroxydihydro-aldrin, which are shown below.



Main metabolite of dieldrin and aldrin

The acute oral toxicity to mammals of the metabolite V was found to be about 1/12 to 1/16 of that of dieldrin. In the next figure the LD_{50} -values (mg/kg mice) of aldrin, dieldrin and metabolite V for oral and intravenous administration are compared in Table 6.

Table 6. Mammalian toxicity of the isolated dieldrin metabolites LD_{50} mg/kg mice.

	Orally	Intravenously
6,7- <i>trans</i> -Dihydroxy-dihydro-aldrin (Main metabolite)	1,250	51
Dieldrin	75~100	15
Aldrin	45	21

Table 5. Comparison of analytical data of metabolite V and synthetical compounds.

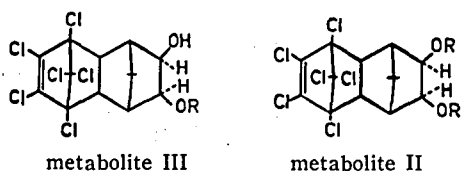
	Metabolite V	Synthetical <i>trans</i> -Dihydroxy-aldrin	Synthetical <i>cis</i> -Dihydroxy-aldrin
Melting point °C	130~131 ^{a)}	132 ^{b)}	217
Infrared spectrum			
OH-Vibrations	3,305K	3,310K	3,230K
CH-Vibrations	2,960+2,905K	2,960+2,910K	2,970+2,915K
R_f -Value ^{b)}	0.16	0.17	0.34

a) Mixed melting point: 132°C (uncorr.)

b) Thin-layer chromatography on silicagel G according to Stahl (E. Merck) mobile phase: benzene/ethylacetate (3:1).

The *trans*-6, 7-dihydroxydihydro-aldrin was also found as main metabolite in the urine of rabbits which had received intravenous administrations of aldrin- $^{14}\text{C}^{21}$.

Two of the other metabolites (metabolites II and III) present in urine in amounts of about 2~4% were found to give the main metabolite by hydrolysis and have probably the following structures.



The unidentified part of the molecule marked R is likely to represent an acyclic-group, this may be concluded from the presence of $\text{C}=\text{O}$ stretching-vibrations in the infrared spectrum.

Metabolites of Telodrin- ^{14}C and Dihydroheptachlor- ^{14}C

After intravenous administration of telodrin- ^{14}C a metabolite was isolated from the excreta of rats 22 . Chromatographical comparison showed that this metabolite was identical to the metabolite isolated from mosquito larvae, namely the lactone,

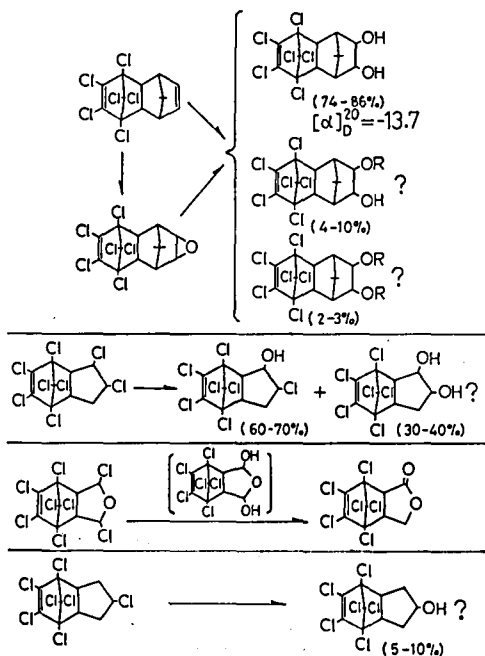


Fig. 15. Metabolites of drin-insecticides.

the acute oral toxicity for mammals of the lactone was about 30 times lower than that of the insecticide applied. One of the sub-metabolites of the dihydroheptachlor found in the excreta of rats proved to be chromatographically identical to synthetical 2-hydroxydihydro-chlordene 23 .

A survey on our identification work of metabolites of drin-insecticides is given in Fig. 15. From this figure a general metabolism and detoxification mechanism is clearly visible: namely dechlorination and hydroxylation. Thus the presence of the lactone as a telodrin metabolite can be easily explained by the acetale intermediate which is unstable and leads to the lactone.

Analysis of the Dieldrin Metabolite by Gas-Liquid Chromatography 24

At the beginning the detection of the main metabolite of dieldrin by gas-liquid chromatography presented some difficulties. In view of the problems encountered in residue analyses, we wanted to find an analytical method enabling us to indicate the metabolite besides the insecticides. Therefore we had to use the same column packings which are also used for the separation and detection of the insecticides, like SE 30 silicone on chromosorb W. It proved that the metabolite will decompose under practical conditions in the GLC at about 200°C. Instead of a peak a flat curve was obtained which was tailing for nearly one hour. This decomposition was also proved by thin-layer chromatograph. When heating the metabolite on silicagel up to 200°C, and after developing the chromatogramme, 3 different compounds resulted on the plate.

Apart from this problem of decomposition the smallest amount of the metabolite detectable by GLC with EC detector is about 500 times that of aldrin or dieldrin. For the practical analyses of residues of aldrin or dieldrin, this means, that—for example—besides 1 ppm of aldrin or dieldrin amounts of the metabolite below 500 ppm could not be detected. Therefore we tried the method which is now usually employed for the gas chromatographical analysis of di- or poly-hydroxy-compounds like carbohydrates. The preparation of trimethylsilyl derivatives: The metabolite dissolved in *n*-hexane was treated with a mixture of pyridine, hexamethyldisilazane and trime-

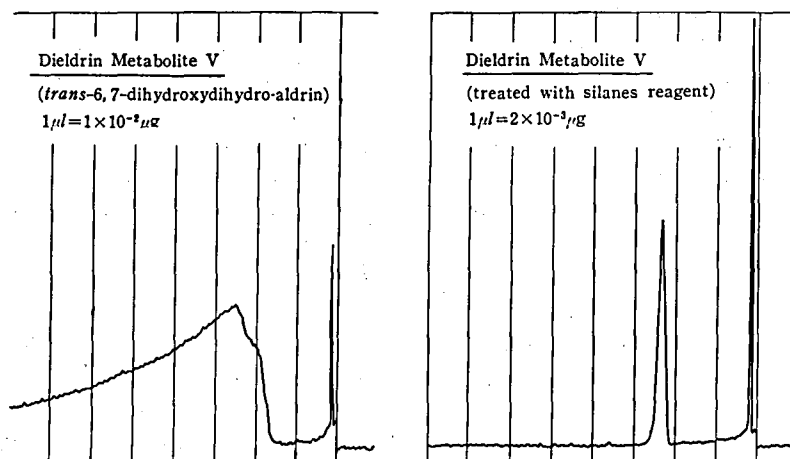


Fig. 16.

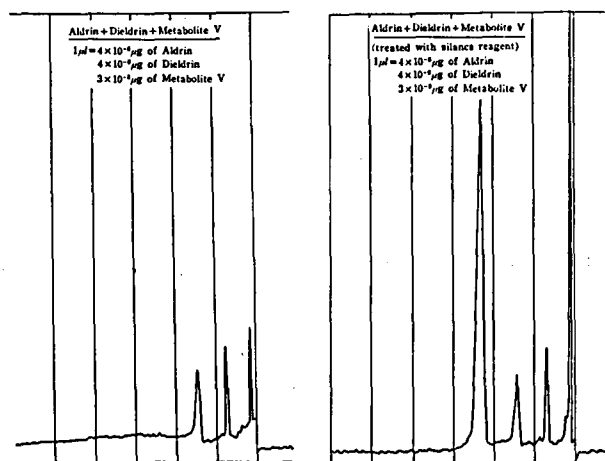


Fig. 17.

thylchlorosilane which were pre-mixed in the ratio 20 : 2 : 1. After standing for one hour at room temperature, water was added to the mixture which was then extracted twice with *n*-hexane. The result of this procedure is shown in Fig. 16. On the left part of the figure a gas chromatogramme of $1 \times 10^{-2} \mu\text{g}$ of the dieldrin metabolite is shown. The right part of the figure shows a gas-chromatogramme of $2 \times 10^{-3} \mu\text{g}$ of the metabolite after treatment with the silanes reagent. The lowest detectable amount of metabolite after this treatment is less than $10^{-4} \mu\text{g}$, this means only about 5~10 times that of aldrin or dieldrin.

In the last figure I would like to demonstrate to you the efficiency of this analytical method

for the detection of the dieldrin metabolite. We prepared a mixture containing $4 \times 10^{-6} \mu\text{g}$ of aldrin, $4 \times 10^{-5} \mu\text{g}$ of dieldrin and $3 \times 10^{-5} \mu\text{g}$ of the dieldrin metabolite in $1 \mu\text{l}$ *n*-hexane. The gas chromatogrammes of this mixture before and after treatment with the silanes reagent are shown in Fig. 17. Summing up, we can say that we have investigated seven different drin-insecticides in three biological test organisms, namely in fungi, mosquito larvae and mammals. All three are able to metabolize the drin-insecticides. Up to now, we could isolate a fairly large number of metabolites which were chromatographically characterized. It could be shown that all of them are more hydrophylic than the starting material and that some of them,

particularly the main products, are identical, no matter whether the tests were carried out with fungi, larvae or mammals.

From the urine of rabbits we could further isolate several metabolites of aldrin, dieldrin and chlordane and elucidate their structures as well as determine their toxicity in mammals.

The long feeding experiments with rats of both sexes seem of particular interest. In both cases they showed that a saturation level is reached after a certain time and that the amount of insecticide remaining in the animal body is excreted after termination of oral application, with a biological half-life period of approximately 11 days for male and 100 days for female animals.

The sex differences in metabolism rate—though not in the kind of metabolism products—also seem of general interest for the research of the metabolism of pesticides by mammals.

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抄 録

昆虫成長阻害・不妊剤, 4-イミダゾリン-2-オン
4-Imidazolin-2-one: An Insect Growth-Inhibitor and Chemosterilant. C. H. Schaefer and C. H. Tieman, *J. Econ. Entomol.* 60, 542 (1967).

4-イミダゾリン-2-オン〔I〕は数種の昆虫に対して強力な増殖ならびに成長阻害剤である。生物活性は飽和同族体2-イミダゾリジノン〔II〕より強い。〔I〕〔II〕共に鳥類、哺乳動物に対して低毒である。不妊剤試験



4-イミダゾリン-2-オン
〔I〕



2-イミダゾリジノン
〔II〕

の結果、イエバエ *Musca domestica* L. の成虫では〔I〕0.05%濃厚ミルク液、または0.002%しょ糖液で不妊効果を示すが、〔II〕は20倍以上の濃度を必要とし